cell proteins, and they occur primarily between regions of complete or partial nucleotide sequence identity within a DNA molecule, such as are present at direct or inverted repeat sequences. Although such deletions could be attributed to instability of the CFTR cDNA in E. coli, computer analysis of the CFTR sequence did not reveal an unusual number of direct and inverted sequence repeats within the published CFTR sequence, thereby indicating that the CFTR cDNA should not be inherently unstable in E. coli.--

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In the Claims

Please cancel Claims 139 to 163 (all of the pending claims) without prejudice.

Please add the following new Claims:

Claim 164. A purified and isolated DNA molecule that includes a nucleotide sequence that encodes human CFTR protein, said encoding nucleotide sequence characterized by the presence therein of a cryptic RNA polymerase promoter sequence, wherein the presence of said DNA molecule in a host E. coli cell transfected therewith causes said cell to be inviable, but wherein said encoding nucleotide sequence is stabilized such that the viability of said cell is improved and said DNA molecule can be stably propagated therein.

Claim 165. A DNA molecule according to Claim 164 wherein the nucleotide sequence thereof that encodes human CFTR protein is a cDNA.

Claim 166. A DNA molecule according to Claim 165 wherein the cryptic promoter sequence in the encoding cDNA thereof is altered by one or more point mutations such that it does not function in E. coli to cause the production of CFTR fragments, and the amino acid sequence encoded by said cDNA is not altered.

Claim 167. A DNA molecule according to Claim 166 containing a T to C mutation at position 936 of the CFTR-encoding cDNA thereof.

Claim 168. A DNA molecule according to Claim 165 wherein the CFTR-encoding cDNA sequence thereof is disrupted by the placement therein of an intervening sequence capable of being spliced from CFTR primary RNA transcript when expressed in a eucaryotic cell, and wherein said intervening sequence is placed downstream from said cryptic promoter therein, and disrupts the translational reading frame of said CFTR-encoding cDNA, or contains one or more stop codons.

Claim 169. A DNA molecule according to Claim 168 wherein said intervening sequence is placed between nucleotides 1716 and 1717 of said CFTR-encoding cDNA.

Claim 170. A purified and isolated DNA molecule that includes a nucleotide sequence that encodes human CFTR protein, said encoding nucleotide sequence characterized by the presence therein of a cryptic RNA polymerase promoter sequence that facilitates production, in a host E. coli cell transfected with said molecule, of CFTR fragments that are toxic to, and cause inviability of, said host

cell, wherein said encoding nucleotide sequence is stabilized to said toxic expression such that the viability of said host cell is improved and said DNA molecule can be stably propagated therein.

Claim 171. A purified and isolated DNA molecule that is patterened on another DNA molecule that includes (1) a nucleotide sequence that encodes human CFTR protein, and (2) a nucleotide sequence within said encoding sequence that defines a cryptic E. coli RNA polymerase promoter, itself capable of directing production of fragments of human CFTR that are toxic when produced in an E.coli cell, and wherein the CFTR-encoding nucleotide sequence of said purified and isolated DNA molecule is stabilized, relative to that in said other DNA molecule, in order to limit expression from said promoter, in E. coli , and production therein of toxic fragments of human CFTR.

Claim 172. In a purified and isolated DNA molecule that includes a nucleotide sequence that encodes human CFTR protein, said encoding nucleotide sequence characterized by the presence therein of a cryptic RNA polymerase promoter sequence that facilitates ~~production~~ expression, in a host E. coli cell transfected with said molecule of CFTR fragments that are toxic to, and cause inviability of, said host cell, the improvement of including therein an encoding nucleotide sequence that is stabilized to said toxic expression, such that the viability of said host cell is improved and said DNA molecule can be stably propagated therein.

Claim 173. A plasmid comprising a DNA molecule according to Claim 164, and one or more regulatory elements operatively linked thereto, wherein said plasmid can be stably maintained and propagated in E. coli cells, and recovered therefrom in purified form.

Claim 174. A plasmid according to Claim 173 wherein a regulatory element thereof is an origin of replication that permits maintenance of said plasmid at about 25 copies or less in a host E. coli cell.

Claim 175. A plasmid according to Claim 173 wherein the cryptic promoter in the CFTR-encoding DNA sequence thereof is altered by one or more point mutations such that it does not function in E. coli to cause production therein of CFTR fragments, and the amino acid sequence of human CFTR encoded by said DNA is not altered.

Claim 176. A plasmid according to Claim 175 wherein said point mutation is a T to C mutation at position 936 of the CFTR-encoding DNA thereof.

Claim 177. A plasmid according to Claim 173 wherein the CFTR-encoding DNA sequence thereof is disrupted by the placement therein of an intervening sequence capable of being spliced from CFTR primary RNA transcript when expressed in a eucaryotic cell, and wherein said intervening sequence is placed downstream from said cryptic promoter in said CFTR-encoding sequence, and disrupts the translational reading frame thereof, or contains one or more stop codons.

Claim 178. A plasmid according to Claim 177 wherein said intervening sequence is placed between nucleotides 1716 and 1717 of said CFTR-encoding DNA.

Claim 179. A plasmid comprising a DNA molecule according to Claim 165, and one or more regulatory elements operatively linked thereto, wherein said plasmid can be stably maintained and propagated in E. coli cells, and recovered therefrom in purified form.

Claim 180. A plasmid according to Claim 179 wherein a regulatory element thereof is an origin of replication that permits maintenance of said plasmid at about 25 copies or less in a host E. coli cell.

Claim 181. A plasmid according to Claim 179 wherein the cryptic promoter in the CFTR-encoding cDNA sequence thereof is altered by one or more point mutations such that it does not function in E. coli to cause production therein of CFTR fragments, and the amino acid sequence of human CFTR encoded by said cDNA is not altered.

Claim 182. A plasmid according to Claim 181 wherein said point mutation is a T to C mutation at position 936 of the CFTR-encoding cDNA thereof.

Claim 183. A plasmid according to Claim 179 wherein the CFTR-encoding cDNA sequence thereof is disrupted by the placement therein of an intervening sequence capable of being spliced from CFTR primary RNA transcript when expressed in a eucaryotic cell, and wherein said intervening sequence is placed downstream from said cryptic promoter in said CFTR-encoding sequence, and disrupts the translational reading frame thereof, or contains one or more stop codons.

Claim 184. A plasmid according to Claim 183 wherein said intervening sequence is placed between nucleotides 1716 and 1717 of said CFTR-encoding cDNA.

Claim 185. A host E. coli cell comprising a plasmid according to Claim 173.

Claim 186. A host E. coli cell comprising a plasmid according to Claim 174.

Claim 187. A host E. coli cell comprising a plasmid according to Claim 175.

Claim 188. A host E. coli cell comprising a plasmid according to Claim 176.

Claim 189. A host E. coli cell comprising a plasmid according to Claim 177.

Claim 190. A host E. coli cell comprising a plasmid according to Claim 178.

Claim 191. A host E. coli cell comprising a plasmid according to Claim 179.

Claim 192. A host E. coli cell comprising a plasmid according to Claim 180.

Claim 193. A host E. coli cell comprising a plasmid according to Claim 181.

Claim 194. A host E. coli cell comprising a plasmid according to Claim 182.

Claim 195. A host E. coli cell comprising a plasmid according to Claim 183.

Claim 196. A host E. coli cell comprising a plasmid according to Claim 184.